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SIMULTANEOUS QUANTIFICATION, METHOD DEVELOPMENT AND METHOD VALIDATION FOR LOSARTAN POTASSIUM AND ENALAPRIL MALEATE IN SOLID DOSAGE FORM BY RP-HPLC

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ABSTRACT
Analytical Method Validation is crucial in pharmaceutical research and manufacturing process. Losartan Potassium and Enalapril Maleate Peaks were well resolved at retention times 9.604 and 2.604 min respectively for 1ml/min flow rate in isocratic mode at a run time of 15 min with Welchrom 250mm x 4.6mm, 5µm column & Ammonium acetate buffer: water: methanol (10:30:60) as mobile phase at 226nm. % Assay values were 99.52% and 99.15% for Losartan potassium and Enalapril maleate respectively. Linearity was produced in the range of 24-72 ppm for Losartan Potassium and 20-60 ppm for Enalapril Maleate with a correlation coefficient of 0.9993 & 0.9998 respectively. The new approach was verified using ICH requirements and found to be specific, sensitive, precise, reliable, and linear. The suggested approach may successfully estimate Losartan Potassium and Enalapril Maleate in marketed formulations.

KEYWORDS: Losartan Potassium, Enalapril Maleate, Method development and validation, ICH guidelines, and RP-HPLC.

1. INTRODUCTION

1.1 DRUG PROFILE OF LOSARTAN POTASSIUM

Losartan is an angiotensin II receptor blocker (ARB), is given to treat hypertension. While angiotensin-converting enzyme (ACE) inhibitors have comparable functions, they frequently produce a cough. Patients who develop a cough after using ACE inhibitors can transition to ARBs such as losartan, which have a cough incidence rate comparable to placebo or hydrochlorothiazide. Losartan potassium oral pills are available, as well as a combo tablet containing both losartan potassium and hydrochlorothiazide. Patients on losartan should have their potassium levels and renal function checked on a regular basis. The FDA approved Losartan on April 14, 1995. The IUPAC name of the medication is (2-butyl-4-chloro-1-{[2'-(2H-1, 2, 3, 4-tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl] methyl}-1H-imidazol-5-yl) methanol is $C_{22}H_{23}ClN_6O$. Losartan is green, with a molecular mass of 422.90 g/mol.[1,8]

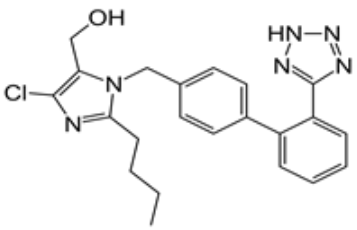


Figure 1: Chemical Structure of Losartan.

1.2 DRUG PROFILE OF ENALAPRIL MALEATE

Enalapril maleate isan oral medication prescribed for managing high blood pressure in adults and children over one month old, whether used alone or in combination with other medications. Enalapril is effective in preventing and treating congestive heart failure in adults. When given as an injection, it helps to control high blood pressure in adults. This drug is classified as an angiotensin-converting enzyme (ACE) inhibitor, working by reducing certain chemicals that narrow blood vessels, thus enhancing blood flow and aiding the heart in pumping blood more efficiently. The IUPAC name for Enalapril Maleate is (2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl] pyrrolidine-2-carboxylic acid. It is white to off-white colour with a molecular weight 376.4467 g/mol and molecular formula $C_{20}H_{28}N_2O_5$ [9-18]. The structure is represented in the below figure.

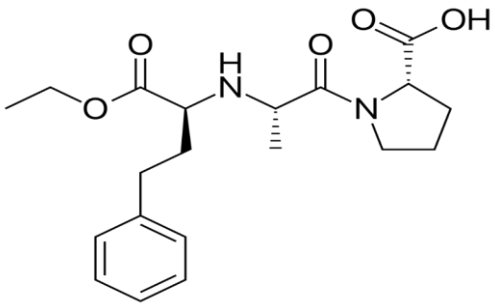


Figure 2: Chemical Structure of Enalapril Maleate.

An extensive literature study [19-30] was carried out and the authors concluded that a very few spectroscopic and liquid chromatographic procedures have been reported. As an outcome, there is a need to design a quick, sensitive, and selective RP-HPLC technique for estimating Losartan Potassium and Enalapril Maleate in bulk and tablet dose forms.

2. MATERIALS AND METHODS

2.1 APPARATUS & CHEMICALS

List of apparatus and chemicals used in this study were tabulated below.

Table 1: List of apparatus.

S.No	Name	Model	Manufacturer
1	HPLC	Waters 2690	ALLIANCE
2	Weighing Balance	SAB 203 L	Scale tech
3	Pipettes, Beakers and Burettes	NA	Borosil Class-A
4	Ultra Sonicator	PSA-10A	DIGITAL PRO

Table 2: List of chemicals.

S.No	Name	Grade	Batch No
1	Water (Milli Q / HPLC Grade water)	HPLC	P24E100596
2	Ammonium acetate	HPLC	J058A24
3	Methanol	HPLC	R276G24

2.2 PREPARATION OF SOLUTIONS

Mobile phase: Ammonium acetate buffer, water & methanol was made in 10:30:60 ratio.

Preparation of buffer: 5 grams of ammonium acetate was accurately weighed and transferred it to a 1000 ml volumetric flask. The volume was made tillmark with water and filtered through a 0.45 µm membrane filter.

Diluent preparation: Water and methanol was mixed in 20:80 ratio.

Standard preparation: Accurately weighed 60 mg of Losartan potassium and 50 mg of Enalapril Maleate and transferred to two separate 100 ml volumetric flasks. 60 mL of diluent was added and sonicated for 5 minutes. The volume was increased to the mark using diluent. Then, 4ml of each solution was put into a 50ml volumetric flask, and the volume was adjusted to the mark using the same dilution.

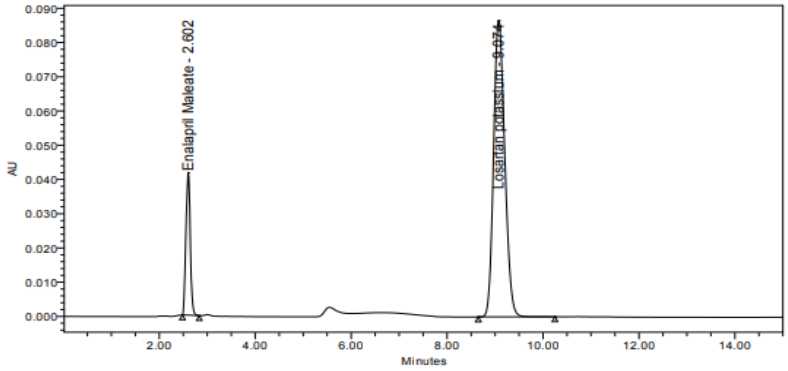
Preparation of Sample solution: Equivalent powders of 60mg and 50 mg were accurately weighed from 20 tablets of COSART - 50 containing Losartan potassium and ENVAS - 10 containing Enalapril Maleate, respectively, and poured to two separate 100 ml volumetric flasks. 60 mL of diluent was added and sonicated for 5 minutes. The volume was increased to the mark using diluent. Then, 4ml of each solution was put into a 50ml volumetric flask, and the volume was adjusted to the mark using the same diluent.

Optimized chromatographic conditions: Several trials were performed in RP-HPLC in isocratic mode and the optimized chromatogram was obtained at 226nm with a flow rate of 1ml/min using Ammonium acetate buffer: water: methanol (10:30:60). Peaks were well separated using Welchrom column of 250mm x 4.6mm, 5µm particle size at an ambient temperature for 15 min run time. The Sample temperature was maintained at 20°± 5°C.

In chromatography, system suitability refers to a set of tests performed to confirm that the chromatographic system (including equipment, electronics, analytical processes, and samples) is operational prior to beginning an analysis. These tests are essential to confirm that the system's performance is acceptable for the intended application and to ensure the reliability of analytical results in accordance to ICH guidelines.[31]

3. RESULTS AND DISCUSSION

3.1 SYSTEM SUITABILITY



	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing	USP Resolution	K Prime
1	Enalapril Maleate	2.602	261269	15.73	41524	3460	1.1		2
2	Losartan potassium	9.074	1400043	84.27	86817	7069	1.2	21	8

Figure 3: System suitability for standard chromatogram.

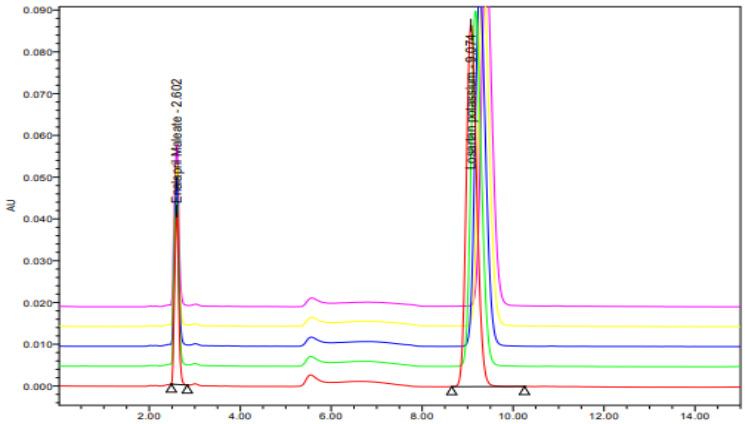


Figure 4: Overlay of System suitability for standard chromatograms.

Table 3: System suitability results.

		Losartan Potassium		Enalapril Maleate	
		Retention Time	Area	Retention Time	Area
1	Mean*	9.253	1404850.0	2.600	262441.9
2	Std. Dev	0.132	5742.7	0.001	1613.8
3	% RSD	1.43	0.4	0.05	0.6

***** Average of five replicate injections.

Discussion: As the theoretical plate value is greater than 2000, Tailing factor is not more than 2.0 and % RSD is not more than 2.0%, the developed method passed the system suitability.

3.2 SPECIFICITY

A high level of specificity ensures that the chromatographic method can accurately and precisely isolate and identify the target compound without interference from other substances within the sample.

Blank

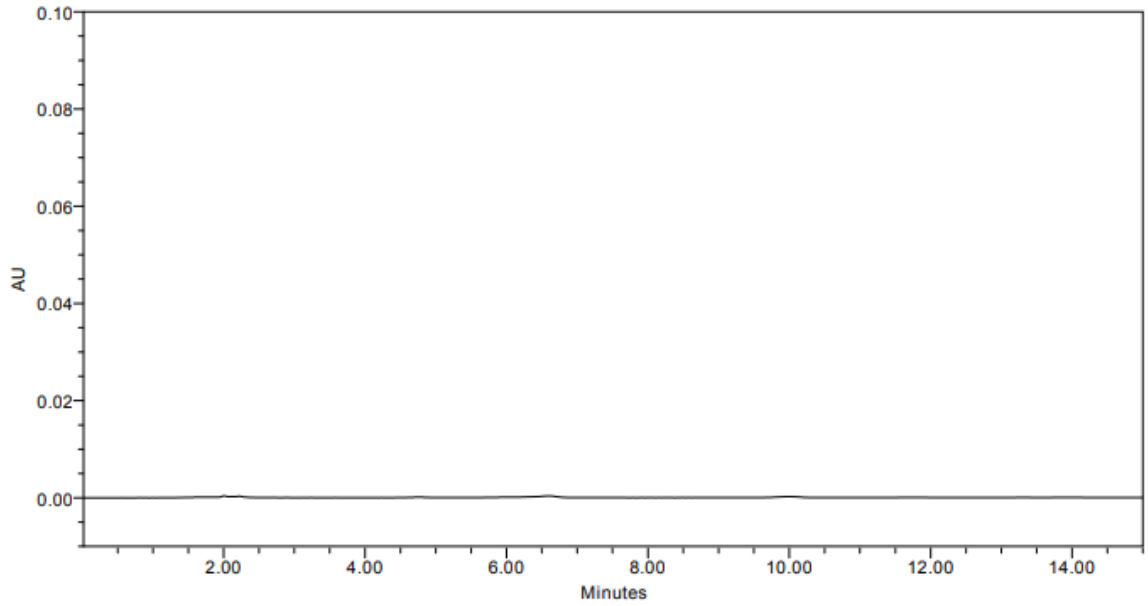


Figure 5: Blank chromatogram.

DISCUSSION

The specificity chromatogram for the blank did not interfere with the main peak and hence, the method is said to be specific.

3.3 ACCURACY

The accurateness of an analytical procedure refers to how closely the test findings match the real value. To assess accuracy, sample solutions at concentrations of 50%, 100%, and 150% of the target analyte are injected into the system, and the percentage recovery is calculated based on the expected values.

Table 4: Results for Accuracy.

S.No	Sample solution concentration*	Losartan Potassium		Enalapril Maleate	
		% RSD	Recovery %	% RSD	Recovery %
1	50%	0.45	99.55%	0.88	99.05%
2	100%	0.05		0.47	
3	150%	0.32		0.59	

***** Average of three replicate injections.

Discussion: The percentage of RSD is not more than 2.0%. As the percentage recovery acceptance criteria is in range of 98.0% - 102.0%, the method is said to be accurate.

3.4 Precision

Consistency and reproducibility refer to obtaining the same results when analyzing the same sample multiple times under identical conditions. Precision quantifies the variability or scatter in these results and is expressed in statistical variables like Standard deviation (SD), Relative Standard Deviation (RSD) & Coefficient of variation (CV).

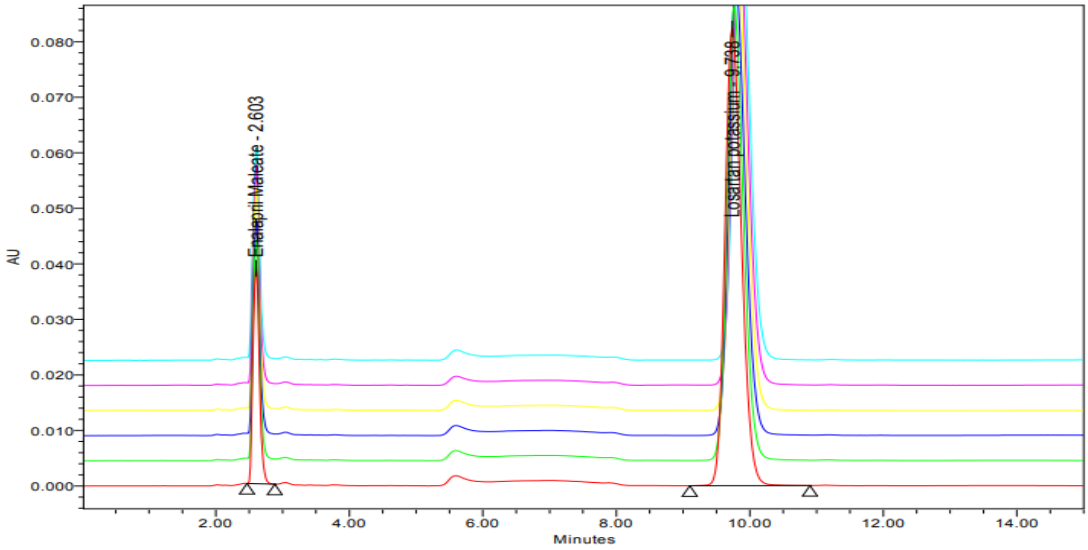


Figure 6: Overlay Precision chromatograms of Losartan potassium and Enalapril maleate.

Table 5: Method precision results for Losartan potassium and Enalapril maleate.

S. No	Peak Name*	Average	SD	% RSD
1	Losartan potassium	1419294	0.26	0.3
2	Enalapril maleate	266656	0.53	0.5

***** Average of six replicate injections

Discussion: The percentage of RSD is not more than 2.0%. As the precision values are within the acceptance criteria for Losartan potassium & Enalapril maleate, the method is said to be precise.

3.5 LINEARITY

Linearity in chromatography refers to the relationship between the concentration or amount of analyte injected and the detector response. A chromatogram demonstrating linearity would show that as the analyte concentration increases or decreases, the detector response (peak area or height) changes proportionally. This characteristic is essential for precise and reliable quantification of substances in samples using chromatographic methods. Five concentrations were prepared each of Losartan Potassium and Enalapril Maleate and each concentration was injected thrice for linearity assessment. Linearity graph was obtained by taking concentration on x-axis and average peak area on y-axis.

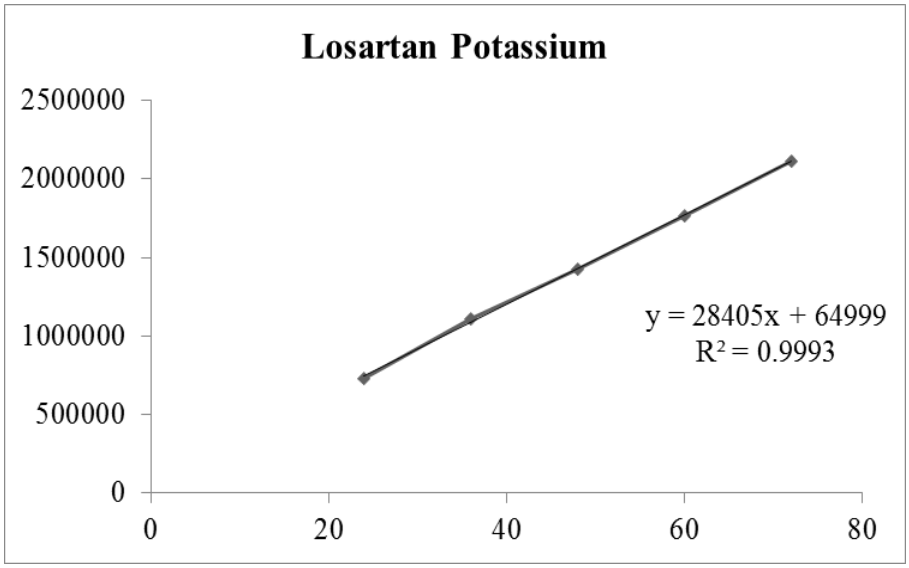


Figure 7: Linearity graph for Losartan Potassium.

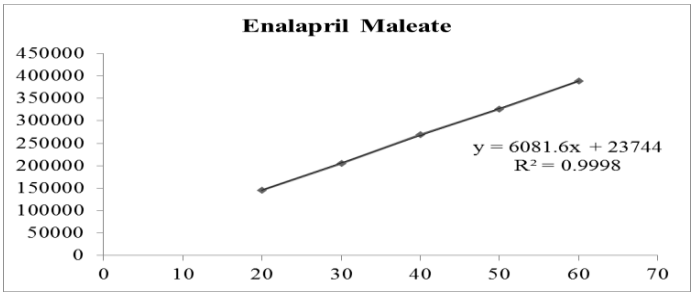


Figure 8: Linearity graph for Enalapril Maleate.

Table 6: Results for linearity.

Losartan potassium		Enalapril Maleate	
Conc. in PPM*	**Peak Area**	**Conc. in PPM***	**Peak Area**
24	732207	20	145216
36	1112124	30	205476
48	1422443	40	269025
60	1765856	50	326585
72	2109667	60	388743
Regression Equation	y = 28405x + 64999	Regression Equation	y = 6081.6x + 23744
Linearity Correlation Coefficient (R^2)	0.9993	Linearity Correlation Coefficient (R^2)	0.9998

***** Average of three replicate injections

DISCUSSION: The R^2 values are within the acceptance criteria i.e NLT 0.99 for Losartan potassium & Enalapril maleate, the method is said to be Linear.

3.6 RANGE

The range is the interval between the greatest and lowest concentrations of the analyte in the sample across which the technique has been shown to be precise, accurate, and linear.

Table 7: Range values for Losartan Potassium & Enalapril maleate.

Percentage of solution	% RSD for Losartan potassium	% RSD for Enalapril maleate
50%	0.40%	0.17%
100%	0.28%	0.77%
150%	0.32%	0.30%

Bracketing standard

Bracketing refers to a strategy in analytical methods where samples are tested at the extremes (upper and lower) of a specified range to ensure accuracy and precision across that entire range.

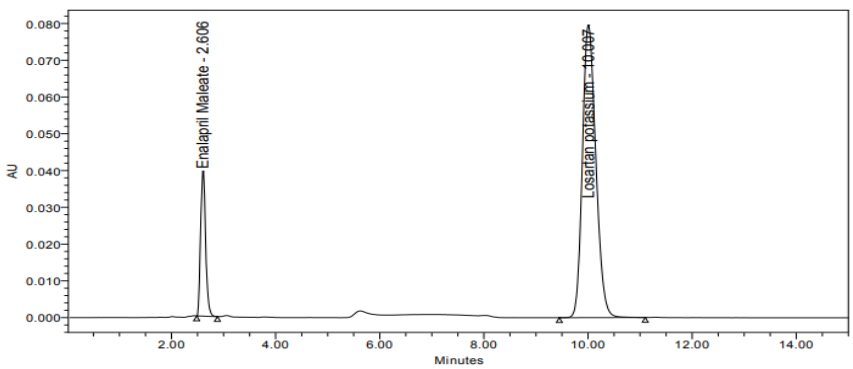


Figure 9: Sample solution Bracketing Standard chromatograms.

Method application to the analysis of Losartan potassium & Enalapril maleate

Assay results are summarized in the below table with the marketed formulations.

Table 8: % Assay results data.

Losartan potassium COSART-50		Enalapril maleate ENVAS-10	
Labeled claim (mg)	**%Assay***	**Labeled claim (mg)**	**%Assay***
50	99.52	10	99.15

***** Average of six replicate injections.

SUMMARY AND CONCLUSION

Parameters	Losartan potassium	Enalapril maleate
% Recovery in Accuracy	99.55%	99.05%
% RSD in Precision	0.3	0.5
Linearity Correlation coefficient	0.9993	0.9998
% Assay	99.52%	99.15%

The study successfully developed and validated a robust RP-HPLC method. This method utilized ammonium acetate buffer, methanol and water in 10:30:60 ratio with isocratic flow, ensuring optimal separation and quantification of both drugs. Chromatographic analysis was performed at a wavelength of 226 nm using 250mm x 4.6mm, 5µm Welchrom column. The system suitability tests which included parameters such as column efficiency, tailing factor, and percentage RSD demonstrated compliance with the established criteria. The technique was verified for specificity, accuracy, precision, linearity, and range, and all fulfilled the ICH approval requirements. Recovery experiments validated the method's precision and accuracy, with percentage recoveries falling between 98% and 102%. The major motivation for validation should be to ensure that all procedures and machinery in the pharmaceutical manufacturing process are used in a manner that ensures the product's safety, integrity, quality, and strength for use by the general population. The approach showed excellent linearity for both medications. The RP-HPLC technique is reliable, accurate, exact, and repeatable, making it appropriate for regular quality control of Losartan Potassium and Enalapril Maleate in tablet dosage forms.

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